PRODUCTION OF BIODEGRADABLE POLY LACTIC ACID FROM PINEAPPLE INDUSTRY WASTEWATER BY *LACTOBACILLUS CASEI* FERMENTATION

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DEDICATION

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ABSTRACT

Pineapple processing industry generates large amount of liquid waste, which contains high concentration of biodegradable organic, suspended solid and high biological oxygen demand (BOD), as well as carbohydrates such as glucose and fructose. The by-products effluent can be a substrate for production of value-added products by microbial fermentation such as biomaterial. However, investigation on the bioconversion of pineapple wastewater to value-added products is still lacking. The objective of this study is to produce biodegradable poly lactic acid (PLA) from pineapple industry waste water by *Lactobacillus casei*. The *L. casei*was cultivated with a medium containing extracted glucose for 24 h. The optimisation of cultural conditions (temperature, pH, inoculum size, nitrogen source) for lactic acid (LA) production was carried out. Purification of LA by reverse osmosis was done. After that, synthesize of PLA was achieved by direct polymerization of pure LA under a nitrogen atmosphere at an optimum time and temperature. Finally, the characterization of PLA was conducted by gel permeation chromatography (GPC), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). A maximum production of 31.6 g/l LA was obtained at pH 6, 37 ℃ and 5% (v/v) of inoculum size. Interestingly, the concentration of pure LA was measured up to 54 g/l after purification. The optimum percentage yield of PLA obtained was 10.4% for 160 ℃ at 12 h and the molecular weight MW was in the range of 1000 Da to 5215 Da. The highly intense band of 2995 was due to CH stretching of the methylene and methyne groups, a range 1716 to 1748 for ester carbonyl group; other significant bands are in region 1118-1360 typical for ester group -CO-O. A clear glass transition (T_g)at 45.3 ℃ was exhibited when heat synthesis at 160℃. The results obtained confirmed the production of PLA. Hence it can be concluded that pineapple wastewater can be used for PLA production by *L. casei* under optimum conditions.

ABSTRAK

Industri pemprosesan nanas menjana sejumlah besar sisa cecair, yang mengandungi organik terdegradasi berkepekatan tinggi, pepejal yang terampai dan oksigen permintaan biokimia yang tinggi (BOD), serta karbohidrat seperti glukosa dan fruktosa. Bahan sisa produk sampingan ini boleh menjadi substrat bagi pengeluaran produk nilai tambah secara penapaian mikroorganism seperti biomaterial. Walau bagaimanapun, penyiasatan mengenai penggunaan air sisa nanas untuk pengeluaran produk nilai tambah adalah terhad. Objektif kajian ini adalah untuk menghasilkan poli LA (PLA) terdegradasi dari air sisa industri nanas oleh Lactobacillus casei. L. casei telah dibiak dengan medium yang mengandungi glukosa terekstrak selama 24 jam. Pengoptimuman keadaan kultur (suhu, pH, saiz inokulum, sumber nitrogen) untuk pengeluaran asid laktik (LA) telah dijalankan. Penulenan LA oleh osmosis berbalik telah dilakukan. Selepas itu, sintesis PLA diperolehi melalui pempolimeran secara langsung LA tulen di bawah keadaan nitrogen pada masa dan suhu yang optimum. Akhirnya, pencirian PLA dilakukan oleh kromatografi penyerapan gel (GPC), spektoskopi Fourier transform inframerah (FTIR), analisis termogravimetrik (TGA) dan ‘differential scanning’ kalorimetri (DSC). Pengeluaran maksimum 31.6 g / l LA diperoleh pada pH 6, 37 ℃ dan 5% (v / v) saiz inokulum. Menariknya, kepekatan LA tulen diukur sehingga 54 g/l selepas penulenan. Hasil peratusan optimum PLA yang diperolehi adalah 10.4 % untuk 160 ℃ pada 12 jam dan berat molekul MW adalah dalam lingkungan 1000 Da hingga 5215 Da. Jalur yang terlalu kuat pada 2995 disebabkan oleh peregangan CH metilena dan metena, dalam lingkungan 1716 hingga 1748 untuk kumpulan karboksil ester; jalur lain yang penting adalah di kawasan 1118-1360 yang tipikal untuk kumpulan ester -CO-O. Peralihan kaca yang jelas (Tg) pada 45.3 ℃ dipamerkan apabila sintesis haba pada 160 ℃. Oleh itu, dapat disimpulkan bahawa air kumbahan nanas boleh digunakan untuk pengeluaran PLA oleh L. casei di bawah keadaan optimum.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

^{o}C	-	Degree Celsius
%	-	percentage
Tm	-	melting temperature
T_g	-	glass transition temperature.
T	-	Time
T_c	-	Melting temperature
Mw	-	Molecular weight
V/V	-	volume per volume
g/dm^{-3}	-	gram per decimeter cubed
mg/l	-	mil gram per liter
MPa	-	mega pascal
Da	-	Dalton
g/ mol	-	gram per mole
cm^{-1}	-	Reciprocal centimeter for wavelength

LIST OF ABBREVIATIONS

LA	-	Lactic acid
MRS	-	De man rogosa and shapes
et al	-	el alia (and other)
g	-	Gram
L	-	Liter
h	-	Hour
mg	-	milligram
OD	-	optical density
rpm	-	revolution per minute
ATCC		American Type Culture CollocationRocckville, Marryland, USA
HPLC	-	high performance liquid Chromatography
LAB	-	LA bacteria
PLA	-	polylactic acid
L.casei	-	*Lactobacillus casei*
DSC	-	differential scanning colorimetry
GPC	-	Gel permeation chromatography
FTIR	-	Fourier transform infrared spectroscopy
OH	-	hydroxyl group
C=O	-	carbonyl group
CH	-	alkyl group
C-O	-	epoxy and oxiyane rings
FAO	-	Food and agriculture organization
SD	-	Standard division
SE	-	Standard error

CHAPTER 1

INTRODUCTION

1.1 Background

The economic actions of many industries such as chemical, petrochemical, agricultural and food have become sources of concern to the world authorities and non-governmental organisations due to the negative effects of these wastes generated by these industries on the environment. For example, pineapple canning industry is amongst the numerous food industries that generate huge amounts of solid and liquids wastes (Vaidya *et al*., 2005). pineapple canning industry is known as one of the many food industries producing large quantities of solid and liquid wastes. The wastewater from this industry contains a high concentration of biodegradable organic material and thus has high biological oxygen demand (BOD) and highly acidic conditions (low pH) (Mohd Zain *et al*., 2010; Abdullah, 2007). Due to the stringent environmental regulations regarding waste disposal, the industry has to provide proper treatment. If these waste discharges to the environment are left untreated, they could cause serious environmental problems such as causing health problems to humans and animals and pollution of the soil (Ren, 2011). The resulting effects of these pollution causes health problems to both humans and animals in the environment. Nevertheless, several economic potentials from using food processing wastes (such as pineapple, orange, mango, apple and so on) are possible, since these wastes act as raw materials in the production of useful and high value-added products. For example, the pineapple wastes are used as microbes feed for biological treatment of wastewater (Jacobsen *et al*., 2000). Furthermore, about 30% of wastes is generated during the canning process of pineapple (Sairi *et al*., 2004). The wastes are produced from various sources during production period has varying characteristics and quantities. The cleaning, separation and pineapple concentrate production are the main sources of liquid waste. The liquid waste of the pineapple industry contains 87% of water, 10% of carbohydrate, 1.7% of fiber, 0.7% of protein, 0.5% of ash, and 0.02% of fat (Sutanto, 2012). In general, the food processing industries contribute about 45% of the total organic pollution from agro-based industries. Therefore, this could be used as inexpensive carbon sources for microbial consortium in the production of lactic acid, acetic acid and vinegar (Jin *et al*., 2005).

LA is an organic compound that is commonly used in the food industry and as substrate for chemical synthesis (Ghaffar *et al*., 2014). It has been revealed that LA can be utilized as organic material for the production of biodegradable polymers; used for packaging in food industries, medical and pharmaceutical products (Wee *et al*., 2006; Busairi, 2010). The growth would come from the LA consumption in chemical applications. For example, the use of poly LA and ethyl lactate is expected to expand 19% per year (Subramanian *et al*., 2015). Currently, the demand of LA was estimated at around 150 million pounds and annual production is between 300,000 to 400,000 tonnes per year (Araya-Cloutier *et al*., 2012).

LA is produced either through chemical synthesis or microbial fermentation. The chemical synthesis produced racemic dextro (-), levo (+), or mixture (DL) of LA as mentioned by Tront *et al*., (2011). Alternatively, LA can be produced through fermentation using specific microbial strain. The most commonly used substrate for submerged LA fermentation is refined sugars, which remained costly (Pal *et al*., 2009). Hence, LA can be produced using cheap substrates (e.g. starch) through two-steps; condition and *Lactobacillus* fermentation processes (Altaf *et al*., 2007). LA is one of the most widely used organic acids in the food industry and is a very common substrate for chemical synthesis. Currently, LA was found to be the most appropriate feedstock monomer for chemical conversion precisely into valuable chemicals such as biodegradable and biocompatible thermoplastics (e.g. polylactic acid). The PLA is one of the thermoplastic aliphatic polyester LA produced using microbial fermentation of renewable agricultural by-products such as food wastes and starch (Södergård *et al*., 2002). The PLA found its application mostly in biomedical fields due to its bioabsorbable properties. However, the recent demand of new polymerization pathways that could permit the economical production of low and high molecular weight PLA, together with the increased in environmental concern of the general public have mandated the widespread application of PLA for goods and packaging purposes (Zhang &Wang, 2008). The biodegradability and sustainable source of PLA make it favorable in reducing the solid waste disposal problems associated with polymeric materials found in the environment. Similarly, its low toxicity and ecofriendly properties made it amongst the most promising material for food packaging and other consumer products (Ren 2009). Therefore, the blending of starch into PLA remained the most encouraging methods for PLA production that could lower the production cost (Zhang *et al*., 2013). However, the main pathway for beginning LA production involved the instantaneous poly condensation reaction (or ring-opening polymerization) of the lactide monomer.

Additionally, polyLA is widely applied as biodegradable and biomedical materials is because of its outstanding properties that include high mechanical strength, transparency, safety and adaptable to hydrolysis. Also, it has good compatibility and usually toxic free. Thus, it can be degradable in human body, which make it suitable for surgical operations (such as implant material) for temporarily healing process (Lan *et al*., 2008).

Currently, there is a need for PLA to possess good mechanical properties and stabilities in order to avoid degradation and loss of its high molecular weight during application in packaging, textile and other plastic materials (Zhang and Wang, 2008). However, its application in the biomedical field (such as joint, drug delivery, systems, and dialysis media) required that the material to be easily biodegradable into LA and subsequently metabolize in the body. It may also have some agricultural application. The environmental degradation caused by the disposal of non-biodegradable plastics will be solved if the uses of these polymers are replaced by PLA. In this study, production of biodegradable PLA from pineapple industry waste water by *Lactobacillus casei* was performed. Characterization of PLA using GPC, FTIR, TGA and DSC were performed. Therefore, this research the production of biodegradable poly lactic acid (PLA) from pineapple industry waste water by *Lactobacillus casei.*

1.2 Problem Statement

Pineapple processing industries are mostly located in tropical region of the world (such as Malaysia, Thailand, Indonesia) and these factories generate large amount of solid and liquid wastes, and with that waste is contain high concentration of biodegradable organic and suspended solid and also high biological oxygen demand (BOD), and contain mainly carbohydrates such as glucose and fructose.

A high level of BOD and COD in pineapple wastes add to further difficulties in disposal (Agus Sutanto, 2012), these wastes are usually prone to microbial spoilage thus limiting further exploitation. Further, the drying, storage and shipment of these wastes is not cost effective and hence efficient, inexpensive and eco-friendly utilization is becoming more and more necessary. The pineapple wastewater residues may cause serious environmental problems, since it accumulates in agro-industrial yards without having any significant and commercial value (Upadhyay *et al*., 2013).

However, if the waste can be transformed into useful products such as organic acid by microbial fermentation, then development of biodegradable and bio-based products that are ecologically and economically sustainable can be attained.

The plastics made using conventional raw materials (e.g. petroleum) takes longer time to degrade into harmless substances compared to those produced from renewable organic materials. Besides, worldwide concern on disposal of plastic wastes produced from petrochemical-beased materials have not been relented due to its environmental impact. Consequently, biodegradable materials, e.g. PLA, have been thoroughly studied in order to substitute the current petro-based polymers. The polymer from renewable sources has several benefits over other polymers; significant energy reduction during production and ability to recycle back to non-toxic, naturally occurring LA through either hydrolysis or alcoholysis. The PLA is primarily obtained from ecofriendly sources which degrade quickly into the environment with virtually very low toxicity. The study on biodegradable polymers recently has gained significant momentum due to its utmost desirability and ecofriendly. However, investigation on the utilisation of pineapple wastewater for production of PLA is scarce. Thus, if wastewater from pineapple can be converted into products such as organic acids; this would derive more value to the waste and in the long run, provide a solution to the environmental problems.

1.3 Objectives

The objectives of the present research are:

i. To characterise the quality of pineapple wastewater.
ii. To extract sugars from pineapple waste.
iii. To produce lactic acid using pineapple wastewater extracted sugar by *L.casei* fermentation.
iv. To isolate lactic acid from the fermented broth.
v. To synthesise poly (lactic acid) polymer by condensation polymerization technique.

1.4 Scope of Study

i. Characterization of physical and chemical properties of pineapple wastewater.
ii. Extraction of sugar content from pineapple wastewater by using rotary evaporator system.
iii. Optimisationof cultural conditions (such as pH, temperature, inoculum size) for high level production of LA.
iv. Purification of LA from the fermentation broth by using reverse osmosis technology.
v. Direct polycondensation of LA for synthesizing PLA under nitrogen condition at different temperature and time.
vi. Characterization of PLA using gel permeation chromatography (GPC), Fourier Transform Infrared spectrophotometer (FTIR), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

1.5 Significance of the Study

This study was carried out to:

i. Obtain a high LA yield from the pineapple waste.

ii. Provide a low-cost carbon source for bacterial growth.
iii. Propose a biodegradable substitute for petroleum-based plastic, from unusable pineapple waste material.

1.6 Outline of the Thesis

The thesis is basically divided into five chapters. The research background, research objective and scope of the study are outlined in Chapter 1. An extensive literature review had been carried out prior to the experimental work. A literature review was conducted to provide background to the research project and this is discussed in detail in Chapter 2.

Chapter 3provides a methodology which includes the steps of the extraction of the carbohydrate, production of LA and the synthesis of PLA. Characterization and recovery of the glucose and optimisation of carbon sources, steps of the fermentation process, and optimisation of pH and temperature as well as polymerization process and the characterization of the synthesised PLA are also discussed.

Chapter 4 consists of a discussion of the results obtained from all the tests conducted including the quality of pineapple wastewater and the whole steps for fermentation process and optimisation of the cultural condition in order to obtain maximum LA. Polymerization steps were performed to produce PLA and characterizations of PLA were determined. Finally, Chapter 5 consists of conclusion and recommendation for future studies.

CHAPTER 2

LITERATURE REVIEW

2.1 Pineapple Industry

2.1.1 Historical Aspects

Pineapple (*Ananas comosus*) is usually a tropical crop grown mostly in Brazil, and other tropical countries of the world. In 1914, the Pineapple Growers Association of Hawaii launched one of the earliest and best rigorous efforts in pineapple improvement. Their major goal was to develop pest and disease resistance species of pineapple. This lead to the development of other agricultural groups in numerous countries (for instance, in Taiwan, Malaysia, Philippines, Cote D'Ivoire, Puerto Rico, Cuba and Australia) to begin hybridization programs for high yield varieties that can adjust to their specific enviromenetal conditions (Guillermo *et al*., 2011; Shari *et al*., 2004). The fruit was introduced to Malaysia by Portuguese in the 16th century and initially planted in Johor and Selangor as a cash crop. After sometimes, the plantation of the fruit was later extended to the peat soil area, particularly in Johor (Hajar *et al*., 2012).

Currently, the world major producers of pineapple include Malaysia, Thailand, Philippines, Indonesia, Hawaii, Ivory Coast, Kenya, Brazil, Taiwan, Australia, India and South Africa. Similarly, countries like Japan, United State of America (USA), Europe Economic Unions (EEU) West Asia and Singapore has high market demand of canned pineapple fruit (http://mpib.gov.my/en/sejarah).

2.1.2 Wide World Production of Pineapple

Pineapple has long remained one of the most prevalent among the non-citrus tropical and subtropical fruits, due to its attractive taste and stimulating sugar-acid stability (Sairi *et al*., 2004). The global production started as early as 1500 when it was spread in Europe and other tropical regions of the world. The most spread specie is *Cayenalisa* variety of pineapple (Smooth Cayenne) which was originated from French Guyana and introduced to Europe. The first canned pineapple was introduced commercially in the late 19th century in Hawaii (Garcia *et al*., 2005).

Pineapple is a member of the Bromeliaceae family (monocotyledons) and consist of about 2000 species with an yearly global production of over 14 million tonnes; it is the eighth most plentifully fruit in the world (Hajar *et al*., 2012). This pommel fruit was perhaps indigenous to Brazil, and it had spread to other parts of tropical America by the time of Columbus who took it to Europe. Currently, the fruit was found to grow all over the tropical and subtropical regions of the world as shown in Figure 2.1.(Dhar *et al*., 2008).

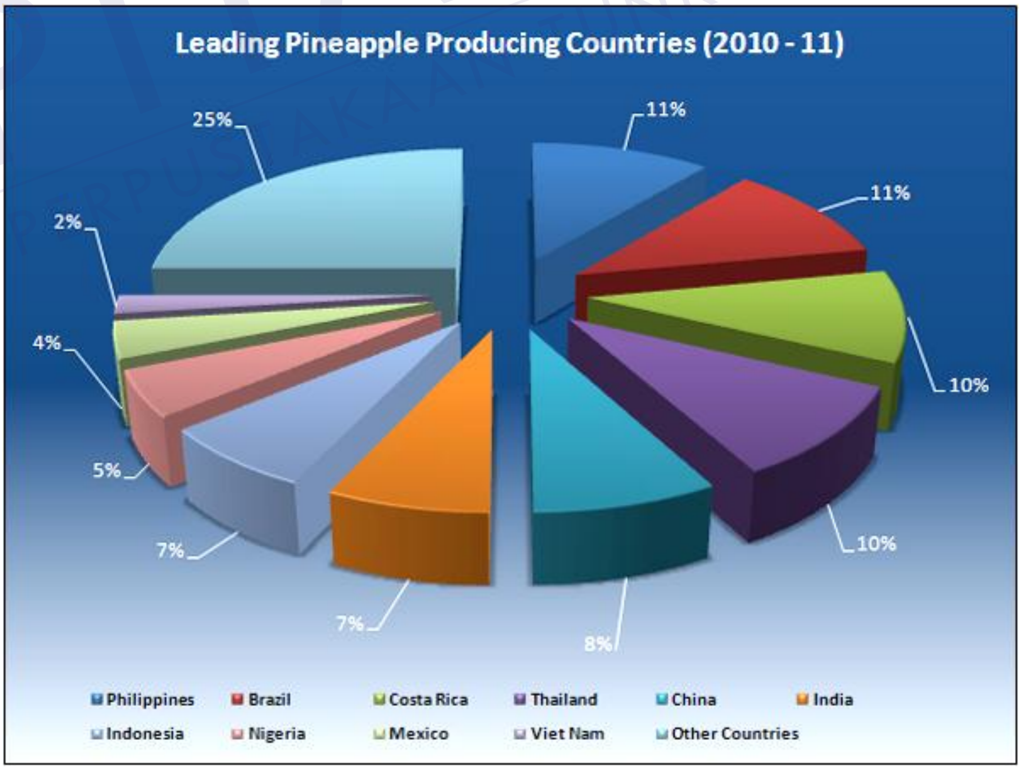

Figure 2.1: World pineapple production (Dhar *et al*., 2008).

The world production of pineapple fruit was 21,865,383tonnes in 2011 and China (the world highest pineapple producer) accounted for about 6.2% (1,351,367 metric tonnes) of the total world's pineapple production (Lu *et al*., 2014). However, a recent study shows that the global pineapple industry is controlled by countries like Costa Rica and the Philippines while countries like Belgium and the Netherland are the major re-exporter of the product. Belgium, France and Germany, Netherlands participate in the world trade by re-exporting the product. Apart from the Philippines, the six chief pineapple producers in the world contribute insignificant quantities to the market of fresh pineapple trade (Table 2.1), (FAO. 2009). The largest exporter of fresh pineapple is Costa Rica (54%), followed by Belgium (10%), Philippines (7%), Netherlands (7%), Ecuador (4%) and the US (3%). The annual export growth recorded between 2005 and 2009 is 30% and 35% of production volume 2.15 to 2.8 million metric tonnes and values $1.1 to $1.5 billion, respectively (Survey, 2012).

Regardless of the rapid growth of the fresh pineapple, the pineapple growing is extremely profitable and have great significance by generating employment and income, and thus, it is broadly exploited throughout the tropical regions and needs serious hand-farming by a countryside man power(Amorim *et al*., 2013).

Table 2.1:Export of fresh pineapple as a percentage share of countries' total production(Amorim *et al*., 2013).

Country	**Percentage**
Netherlands	88.9
Ivory Coast	82.2
Belgium	78.9
Costa Rica	67.9
Honduras	61.3
France	52.2
Ghana	36.4
Malaysia	14.7
Nicaragua	12.9
United State	12.5
Germany	11.2
Philippines	8.9
Ecuador	5.1
México	4.7
Brazil	1.2
Thailand	0.2
China	0.2
India	0.1

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